Shamsaldeen, Y., Culliford, L., Clout, M., James, A., Ascione, R., Hancox, J., & Marrion, N. (2019). Role of SK channel activation in determining the action potential configuration in freshly isolated human atrial myocytes from the SKArF Study. *Biochemical and Biophysical Research Communications*, *512*(4), 684-690. https://doi.org/10.1016/j.bbrc.2019.03.074

# Role of SK channel activation in determining the action potential configuration in freshly isolated human atrial myocytes from the SKArF Study

Yousif A Shamsaldeen, Lucy Culliford[+], Madeleine Clout[+], Andrew F. James, Raimondo Ascione*, Jules C. Hancox & Neil V. Marrion

School of Physiology, Pharmacology & Neuroscience, University of Bristol, Bristol, BS8 1TD, UK.

[+]Clinical Trials and Evaluation Unit, Bristol Trials Centre, Bristol Medical School, University of Bristol, Bristol, UK

*Translational Biomedical Research Centre, Faculty of Health Sciences, University of Bristol

**Author for correspondence**:

Prof. Neil Marrion, School of Physiology, Pharmacology & Neuroscience, University of Bristol, Bristol, BS8 1TD.

Tel: +441173311401

Email: N.V.Marrion@bristol.ac.uk

**Inhibition of SK channel function is being pursued in animal models as a possible therapeutic approach to treat atrial fibrillation (AF). However, the pharmacology of SK channels in human atria is unclear. SK channel function is inhibited by both apamin and UCL1684, with the former discriminating between SK channel subtypes. In this proof-of-principle study, the effects of apamin and UCL1684 on right atrial myocytes freshly isolated from patients in sinus rhythm undergoing elective cardiac surgery were investigated. Outward current evoked from voltage clamped human atrial myocytes was reduced by these two inhibitors of SK channel function. In contrast, membrane current underlying the atrial action potential was affected significantly only by UCL1684 and not by apamin. This pharmacology mirrors that observed in mouse atria, suggesting that mammalian atria possess two populations of SK channels, with only one population contributing to the action potential waveform. Immuno-visualization of the subcellular localization of SK2 and SK3 subunits showed a high degree of colocalization, consistent with the formation of heteromeric SK2/SK3 channels. These data reveal that human atrial myocytes express two SK channel subtypes, one exhibiting an unusual pharmacology. These channels contribute to the atrial action potential waveform and might be a target for novel therapeutic approaches to treat supraventricular arrhythmic conditions such as atrial fibrillation.**

**Keywords:** atria, human, action potential, SK channel, pharmacology, heteromer

**Acknowledgements:** The authors would like to thank the SKARF study team and research nurses at University Hospitals Bristol NHS Foundation Trust involved in the recruitment, coordination, and data collection for this study and most of all the patients consenting to take part. This study was delivered in collaboration with the Clinical Trials and Evaluation Unit (CTEU), a UKCRC registered clinical trials unit which, as part of the Bristol Trials Centre.

**Funding:** The trial was funded by a Bristol Heart Foundation (BHF) grant (BHF PG/16/37/) to Marrion, Hancox, James and Ascione. The study was also supported by the National Institute for Health Research (NIHR) Bristol Biomedical Research Centre at University Hospitals Bristol NHS Foundation Trust and the University of Bristol. The views expressed in this publication are those of the author(s) and not necessarily those of the NHS, the National Institute for Health Research or the Department of Health and Social Care.

## Introduction

Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia [1] and a major health issue that affects approximately 2% of the population of Europe and the USA [2, 3]. Aging is a major risk factors for AF, and it is estimated that the prevalence of AF will double in the next 50 years [4]. Among the numerous consequences of AF are stroke, systemic embolization and heart failure [4]. The mechanistic cause of stand-alone AF is still unknown, although there are additional predisposing conditions such as the presence of mitral valve regurgitation [5] or the undertaking of cardiac surgery [6-8]. This uncertainty and variability has led to treatments that have a wide range of pharmacological actions [9], which are associated with a wide spectrum of cardiac side effects that include impaired left ventricular contractile function or serious arrhythmias such as atrioventricular block and QT prolongation (torsades de pointes) [9].

Atrial action potential duration is prolonged by inhibition of SK channel function [15, 16, 20]. Three subtypes of SK channel subunit have been cloned, termed SK1-3 [10], with both homomeric and heteromeric channels being able to assemble [10, 11]. There is a greater level of expression of SK channel subunits in human atria compared with ventricles, with higher levels of mRNA encoding for SK2 and 3 than for SK1 in atria [12]. The gene encoding SK3 was identified in a meta-analysis to be a common genetic variant present in

AF [13]. The association between clinical AF and SK channel expression was confirmed in studies of larger populations [14]. Induced AF in rats [15], horse [16] and pig [17] is terminated by SK channel inhibition, while co-inhibition of sodium current and SK channel function terminated AF in guinea pig [16]. However, the pharmacology of SK inhibition in atria is not straightforward. SK channel currents are characterised by high sensitivity to the toxin apamin, which is found in the venom of the Honey Bee [10], which can discriminate between SK channel subtypes [18]. Small molecule inhibitors of SK channel function are available but inhibit all SK channel subtypes with equal potency [18]. However, the ability of inhibition of SK channel function to terminate induced AF is not generally observed with apamin [19]. This has led to investigators using small molecule inhibitors that provide a clear effect, including UCL1684, NS8593 and ICAGEN [12, 16]. For example, inhibition of SK channel current by NS8593 or ICAGEN prolonged the human atrial action potential duration (APD) [12]. Study of SK channel function in mouse atria revealed that inhibition of SK channel function by UCL1684 prolonged the APD, but this effect was not reproduced by apamin [20]. In contrast, whole-cell voltage clamp-evoked outward currents were reduced by both apamin and UCL1684 [20]. It was proposed that mouse atria possess two populations of SK channels: apamin-sensitive homomeric SK2 channels and apamin-insensitive heteromeric SK2-SK3 channels. Each population is activated differently, with both populations of channel being activated during whole-cell voltage-clamp recordings, but only the apamin-insensitive channels contribute to repolarization of the atrial action [20]. It is important to resolve which SK channel subtypes are present in human atria, as it has been proposed that the SK channel contributing to action potential repolarization is a therapeutic atrial-selective target for antiarrhythmic drug therapy [12]. We report that whole-cell voltage clamp current recorded from acutely isolated human atrial myocytes were reduced in amplitude by both apamin and UCL1684. In contrast, membrane current underlying the atrial action potential was only significantly affected by UCL1684 and not apamin. These data are in accord with those reported for mouse atria [20] and indicate that both human and

mouse possess two populations of SK channel, with only one being a therapeutic target to affect action potential configuration.

## Materials and Methods

*Patient selection and human atrial sample harvesting*

Human atrial samples were collected from 18 patients undergoing cardiac surgery at the Bristol Heart Institute, University Hospital NHS Foundation Trust (IRAS number 211164) during 2017-18 and recruited to the SKArF study. The study received approval from the West of Scotland Research Ethics Committee 5 (Reference 16/WS/0172). Informed consent was received from all participants. Under general anaesthesia, following chest opening and heparinisation, and before commencing cardiopulmonary bypass, the tip of the right atrial appendage was removed as surplus tissue and plunged into pre-oxygenated sterile ice-cold cardioplegic solution of composition: (mM): glucose, 140; HEPES free acid, 10, adenosine, 5, mannitol, 10, $KH_2PO_4$, 50, and $MgSO_4$, 8mM; pH 7.4 (KOH) (284 mOsm). Tissue was transferred into calcium-free ($Ca^{2+}$-free) isolation solution of composition (mM): NaCl, 130; KCl, 5.4; Glucose, 10; HEPES free acid, 10; $NaH_2PO_4$, 0.4; $MgCl_2$, 1.4; taurine, 20; creatine, 10; pH 7.6 (NaOH) (285 mOsm) for cleaning and cutting into chunks. The chunks were then transferred to fresh oxygenated warm $Ca^{2+}$-free isolation solution and incubated for 3 minutes at 37$^{o}$C while shaking at 100 rpm. The solution was then decanted gently through a nylon filter sheath (200 µm) and replaced with new oxygenated warm $Ca^{2+}$-free isolation solution for 3 minutes. Chunks were then placed in warm EGTA (8 µM)-containing $Ca^{2+}$-free isolation solution and incubated for a further 3 minutes. Tissue was transferred back into $Ca^{2+}$-free isolation solution supplemented with bovine serum albumin (BSA) (1% w/v), collagenase (0.2 mg/ml) and proteinase (0.4 mg/ml) and incubated for 10 minutes at 37$^{o}$C in a shaking water bath, with $CaCl_2$ (20 µM) added after 10 minutes and chunks were incubated for a further 35 minutes at 37$^{o}$C. Tissue was then washed with fresh warm oxygenated $Ca^{2+}$-free isolation solution and then placed in $Ca^{2+}$-free isolation solution supplemented with $CaCl_2$ (20 µM), BSA (1%) and collagenase (0.2 mg/ml) at 37$^{o}$C to be

sampled at 5 minutes intervals until the appearance of striated rod-shaped cardiomyocytes. Enzyme activity was then stopped by addition of foetal bovine serum (1%) and cells transferred into 35 mm petri-dishes containing KB storage solution of composition (mM): L-glutamic acid, 90; KCl, 30; Glucose, 20; HEPES free acid, 10; EGTA, 1; sodium pyruvate, 5; $NaH_2PO_4$, 0.4; $MgCl_2$, 5; taurine, 20; creatine, 5; succinic acid, 5; sodium butyric acid, 5; $Na_2ATP$, 2; pH 7.4 (KOH) (280 mOsm).

*Electrophysiology*

Isolated atrial myocytes were continuously perfused with an external solution containing (mM): NaCl, 144; Glucose, 10; HEPES free acid, 10, KCl, 2.5; $MgCl_2$, 1.2, $CaCl_2$, 2.5, pH 7.4 (NaOH) (300 mOsm). Evoked currents were recorded using the whole-cell technique with electrodes pulled from KG-33 glass and containing (mM): potassium aspartate, 130; HEPESNa, 5; NaOH, 3; KCl, 20; $CaCl_2$, 50 nM; $MgCl_2$, 1.5; $Na_2ATP$, 2.5; pH 7.4 (NaOH) (311 mOsm). Cells were voltage clamped at -80 mV at room temperature, using an Axopatch 200A patch-clamp amplifier (Molecular Devices (UK) Ltd, Berkshire, UK). Currents were filtered with an 8-pole Bessel filter (Frequency Devices, IL, USA) at a cut-off frequency of 1 kHz and sampled at 10 kHz using Pulse software (HEKA, Lambrecht/Pfalz, Germany). Electrode resistances were 4-7 MΩ and whole-cell capacitance and series resistance were compensated for by 20%–50%.

Apamin (Sigma Aldrich, UK) and charybdotoxin (Tocris Biosciences, Bristol, UK) were dissolved in water and stored at a concentration of 100 µM at -20 $^{o}$C, whereas UCL1684 (Tocris Biosciences, Bristol, UK) was dissolved in DMSO and stored at a concentration of 100 µM. Aliquots were thawed and added to external bath solution to give required concentrations.

All electrophysiology data was analysed using Pulse (HEKA) and Origin (version 9, Originlab Corp, MA, USA) and Prism (version 7, Graphpad Software Inc, CA, USA). Statistical significance was determined by using either a t-test or a two-way ANOVA with Bonferroni post-hoc analysis.

*Immunocytochemistry*

Freshly isolated atrial myocytes were fixed and permeabilized by incubating with ice-cold acetone 100% for 7 minutes [6], followed by blocking the cells with horse serum (2.5%) (Vector Laboratories: DK-8828) for 30 minutes. Cells were then incubated overnight at room temperature with mouse anti-SK2 antibody (Biorbyt: orb333955) and rabbit anti-SK3 antibody (Biorbyt: orb157735), added as a 1:200 dilution in blocking solution of composition (mM): phosphate buffer saline, bovine serum albumin (Sigma Aldrich, A9418) (1%), and foetal calf serum (Thermo Fisher Scientific, 10500-064) (2%) [20, 21]. Cells were then washed twice with phosphate buffered saline (PBS) and incubated for 3 hours with secondary antibodies in a premixed double labelling solution of anti-rabbit IgG (red DyLight 594) and anti-mouse IgG (green DyLight 488) (Vector Laboratories: DK-8828). Finally, cells were washed three times with PBS before being mounted with mounting medium with DAPI (Vector Laboratories: H-1200). The cells were examined using a Leica AOBSSP2 confocal laser scanning microscope (Leica, Solms, Germany). The objective used was a 40x (NA 1.4) oil immersion lens. Brightness and contrast were increased uniformly for each experiment using Volocity software (PerkinElmer, Waltham, MA). Co-localisation of SK2 and SK3 subunits is quantified using Mander's coefficient, where the emission intensities from each secondary antibody fluorophore are ratioed with the intensity distribution of co-localising objects and the total intensity of the respective components of the image [22].

**Results**

Patient baseline characteristics and risk profile are shown in Table 1. Tissue was obtained from 18 patients (14 male, 4 female; mean age 67).

**Activation of SK channel current in adult human atrial myocytes**

Visualised striated, rod shaped human atrial myocytes were whole-cell voltage clamped and current was evoked by step depolarizations from a holding potential of -80 mV. Evoked outward current increased in amplitude with depolarization, displaying typical outward rectification (**Figure 1A**). Extracellular application of either apamin (100 nM) or UCL 1684 (100 nM) reduced the amplitude of evoked current at all membrane potentials (**Figure 1B**). Apamin inhibited the current by 14.3 ± 2.7% at +10 mV (4 patients, n=8 cells), with current reduced from 500 ± 100 pA to 430 ± 100 pA (P=0.000008). A similar reduction in outward current was observed with the small organic molecule inhibitor of SK current UCL1684, producing a reducing current amplitude at +10 mV by 17.5 ± 2.8% (4 patients, n=10 cells), from 500 ± 90 pA to 420 ±90 pA (P=0.00000014) (**Figure 1B**). The effect of apamin and UCL1684 were comparable (P= 0.91). These data indicate the presence of functional SK channels in human atrial myocytes under our experimental conditions, as reported in mouse [20].

**Inhibition of SK channel current reduces the charge movement underlying the human atrial action potential**

An unusual pharmacology has been reported for the mouse action potential, with the duration being prolonged by UCL1684 but not by apamin [20]. It is important to determine whether this unusual pharmacology is present in human atria. We elected to use action potential clamp of the whole-cell configuration to isolate the ionic currents underlying the human atrial myocyte action potential. The effect of drug was determined by integration of the waveform to provide an estimate of total charge that was passed during the action potential voltage waveform. Application of UCL1684 (100 nM) decreased the charge transfer by 20.6 ± 5.3 % (n=3) (P<0.01). In contrast, application of apamin (100 nM) had

very little effect on charge transfer, with it decreasing by 5.6 ± 0.2 % (n=3) (P=0.97). The effect of UCL1684 was significantly different from any effect of apamin (P=0.05). The effect of these two SK current inhibitors can be seen when the drug-sensitive trace is produced and compared, as shown in **Figure 2**. The UCL1684-sensitive trace shows a waveform that suggests activation of SK channels early in the action potential waveform, while the current inhibited by apamin is essentially flat. These data are similar to those reported for mouse atria, where apamin was without a significant effect on the action potential duration but UCL1684 prolonged the waveform [20]. It was proposed that a heteromeric channel comprising SK2 and SK3 subunits exhibited this unusual pharmacology [20].

**Immuno-visualization of SK2 and SK3 subunits in human atrial myocytes**

The presence of SK channel protein in human atria has been reported, with some evidence that expression is highest in atrial myocytes [23-25]. Our pharmacological data would suggest that a heteromer between SK2 and SK3 subunits might comprise the SK channel that contributes to action potential repolarization [20]. Human atrial myocytes were fixed, permeabilised and incubated with primary antibodies directed against SK2 and 3 subunits. Immuno-visualization showed SK2 and SK3 were expressed in acutely isolated human atrial myocytes, with labelling concentrated at cell periphery (**Figure 3A**). The intensity of labelling was similar for both SK2 and SK3 subunits, suggesting that both subunits were expressed at approximately the same level uniformly across human atrial myocytes (**Figure 3B**). Examination of colocalization showed that 93% of SK3 subunits were overlapped with SK2 subunits, and 94% of SK2 subunits were overlapped with SK3 subunits (**Figure 3C**). These data suggest that both SK2 and SK3 subunits are expressed in human atrial myocytes, with most subunits colocalised to suggest that heteromeric channels are formed. Expressed heteromeric SK2-SK3 channel current is inhibited by UCL1684, but insensitive to inhibition by apamin [20]. These data are consistent with the pharmacology of the current underlying repolarization of the atrial action potential, where only UCL1684 affected the current evoked

in action potential clamp (**Figure 2**). Together, it is proposed that activation of heteromeric SK2-SK3 channels contributes to repolarization of the human atrial myocyte action potential.

## Discussion

Expression of SK1-3 subunits has been demonstrated in a number of species, including human, horse, pig, rat and mouse [10, 16-18, 20]. Activation of SK channels has been demonstrated to contribute to action potential repolarization in human and mouse atrial myocytes [20, 26]. It is unusual however, that apamin is much less effective than small organic molecule inhibitors [20, 26]. This unusual pharmacology is apparent in reports suggesting that inhibition of SK channel function terminates induced atrial fibrillation (AF) *in vivo* in horse [27], rat [19], and pig [17], and *in vitro* in guinea pig [28] and rat [29]. Termination of AF has been demonstrated with small organic molecules such as NS8593 and UCL1684, but apamin was significantly less effective when tested [19, 30]. Apamin acts as a negative allosteric modulator, binding to both the outer pore turret and the S3-S4 extracellular loop to inhibit channel activity [19, 27]. In contrast, small organic molecule inhibitors such as UCL1684 only bind the outer pore turret, with it being proposed that the lack of binding to the extracellular loop prevents these molecules from selecting between SK channel subtypes [19]. UCL1684 has been found to be very selective for SK channels, not affecting a number of ion channel currents that underlie the atrial action potential [20]. SK channel subunits of different identities can co-assemble to form functional heteromeric channels that display atypical pharmacology [11, 20]. For example, expressed heteromeric SK2-SK3 channel current was insensitive to apamin but inhibited by UCL1684 [20], which matched data showing that the mouse atrial action potential duration was prolonged by UCL1684, but not by apamin [20]. We observed a similar pharmacology with acutely dissociated human atrial myocytes (**Figure 2**). However, in both mouse [20] and human (this study) apamin reduced outward current under voltage clamp (**Figure 1**). The concentration-inhibition relationship for apamin in mouse atrial myocytes indicated a

sensitivity reflecting inhibition of homomeric SK2 channels [20]. These data suggest that both human and mouse atrial myocytes express two populations of SK channels, homomeric SK2 and heteromeric SK2-SK3 channels [20].

It has been proposed that inhibition of SK channel current is a therapeutic approach for the treatment of AF [12]. Our study suggests that only activation of SK2-SK3 heteromeric channels contributes to action potential repolarization in human (Figure 2) and mouse atrial myocytes [20]. The function of the discreet population of homomeric SK2 channels [20] is unknown. It is therefore possible that there is a limited distribution of heteromeric SK2-SK3 channels in the periphery, as expression of SK3 protein is limited to heart [12] and skeletal muscle [31, 32] and SK2 is not expressed in skeletal muscle [32]. This raises the possibility that targeting a SK2-SK3 heteromer would give a selective therapeutic effect in halting AF in otherwise healthy subjects [20] with less side effects. Importantly, it is clear that like mouse atria, human atrial myocytes utilise a SK channel with unique pharmacology to repolarise the action potential that allows mouse to be a suitable translational model for future studies aiming at developing more effective pharmacological approaches to AF.

**Conflict of interest statement**

None declared.

**References**

[1] A.J. Workman, K.A. Kane, A.C. Rankin, Cellular bases for human atrial fibrillation, Heart Rhythm, 5 (2008) S1-S6.
[2] M. Zoni-Berisso, F. Lercari, T. Carazza, S. Domenicucci, Epidemiology of atrial fibrillation: European perspective, Clinical epidemiology, 6 (2014) 213.
[3] G.Y. Lip, C.M. Brechin, D.A. Lane, The global burden of atrial fibrillation and stroke: a systematic review of the epidemiology of atrial fibrillation in regions outside North America and Europe, Chest, 142 (2012) 1489-1498.

[4] Developed with the special contribution of the European Heart Rhythm Association, Endorsed by the European Association for Cardio-Thoracic Surgery, Authors/Task Force Members, A.J. Camm, P. Kirchhof, G.Y. Lip, U. Schotten, I. Savelieva, S. Ernst, I.C. Van Gelder, Guidelines for the management of atrial fibrillation: the Task Force for the Management of Atrial Fibrillation of the European Society of Cardiology (ESC), European heart journal, 31 (2010) 2369-2429.
[5] F. Grigioni, J.-F. Avierinos, L.H. Ling, C.G. Scott, K.R. Bailey, A.J. Tajik, R.L. Frye, M. Enriquez-Sarano, Atrial fibrillation complicating the course of degenerative mitral regurgitation: determinants and long-term outcome, Journal of the American College of Cardiology, 40 (2002) 84-92.
[6] E. Akintoye, F. Sellke, R. Marchioli, L. Tavazzi, D. Mozaffarian, Factors associated with postoperative atrial fibrillation and other adverse events after cardiac surgery, The Journal of thoracic and cardiovascular surgery, 155 (2018) 242-251. e210.
[7] V.D. Bruno, R. Ascione, Postoperative atrial fibrillation: Still in search of truth or a neglected complication?, The Journal of thoracic and cardiovascular surgery, 155 (2018) 236-237.
[8] R. Ascione, M. Caputo, G. Calori, C.T. Lloyd, M.J. Underwood, G.D. Angelini, Predictors of atrial fibrillation after conventional and beating heart coronary surgery: a prospective, randomized study, Circulation, 102 (2000) 1530-1535.
[9] P. Kirchhof, S. Benussi, D. Kotecha, A. Ahlsson, D. Atar, B. Casadei, M. Castella, H.-C. Diener, H. Heidbuchel, 2016 ESC Guidelines for the management of atrial fibrillation developed in collaboration with EACTS, EP Europace, 18 (2016) 1609-1678.
[10] M. Köhler, B. Hirschberg, C. Bond, J.M. Kinzie, N. Marrion, J. Maylie, J. Adelman, Small-conductance, calcium-activated potassium channels from mammalian brain, Science, 273 (1996) 1709-1714.
[11] T.W. Church, K.L. Weatherall, S.A. Corrêa, D.L. Prole, J.T. Brown, N.V. Marrion, Preferential assembly of heteromeric small conductance calcium-activated potassium channels, European Journal of Neuroscience, 41 (2015) 305-315.
[12] L. Skibsbye, C. Poulet, J.G. Diness, B.H. Bentzen, L. Yuan, U. Kappert, K. Matschke, E. Wettwer, U. Ravens, M. Grunnet, Small-conductance calcium-activated potassium (SK) channels contribute to action potential repolarization in human atria, Cardiovascular research, 103 (2014) 156-167.
[13] P.T. Ellinor, K.L. Lunetta, N.L. Glazer, A. Pfeufer, A. Alonso, M.K. Chung, M.F. Sinner, P.I. De Bakker, M. Mueller, S.A. Lubitz, Common variants in KCNN3 are associated with lone atrial fibrillation, Nature genetics, 42 (2010) 240.
[14] P.T. Ellinor, K.L. Lunetta, C.M. Albert, N.L. Glazer, M.D. Ritchie, A.V. Smith, D.E. Arking, M. Müller-Nurasyid, B.P. Krijthe, S.A. Lubitz, Meta-analysis identifies six new susceptibility loci for atrial fibrillation, Nature genetics, 44 (2012) 670.
[15] J.G. Diness, U.S. Sørensen, J.D. Nissen, B. Al-Shahib, T. Jespersen, M. Grunnet, R.S. Hansen, Inhibition of small conductance Ca2+-activated K+ channels terminates and protects against atrial fibrillation, Circulation: Arrhythmia and electrophysiology, (2010) CIRCEP. 110.957407.
[16] J.E. Kirchhoff, J.G. Diness, M. Sheykhzade, M. Grunnet, T. Jespersen, Synergistic antiarrhythmic effect of combining inhibition of Ca2+-activated K+ (SK) channels and voltage-gated Na+ channels in an isolated heart model of atrial fibrillation, Heart Rhythm, 12 (2015) 409-418.
[17] J.G. Diness, L. Skibsbye, R. Simó-Vicens, J.L. Santos, P. Lundegaard, C. Citerni, D.R.P. Sauter, S.H. Bomholtz, J.H. Svendsen, S.-P. Olesen, Termination of vernakalant-resistant atrial fibrillation by inhibition of small-conductance Ca2+-activated K+ channels in pigs, Circulation: Arrhythmia and Electrophysiology, 10 (2017) e005125.
[18] K.L. Weatherall, S.J. Goodchild, D.E. Jane, N.V. Marrion, Small conductance calcium-activated potassium channels: from structure to function, Progress in neurobiology, 91 (2010) 242-255.
[19] L. Skibsbye, J.G. Diness, U.S. Sørensen, R.S. Hansen, M. Grunnet, The duration of pacing-induced atrial fibrillation is reduced in vivo by inhibition of small conductance Ca2+-activated K+ channels, Journal of cardiovascular pharmacology, 57 (2011) 672-681.

[20] J.M. Hancock, K.L. Weatherall, S.C. Choisy, A.F. James, J.C. Hancox, N.V. Marrion, Selective activation of heteromeric SK channels contributes to action potential repolarization in mouse atrial myocytes, Heart Rhythm, 12 (2015) 1003-1015.
[21] Y.A. Shamsaldeen, R. Ugur, C.D. Benham, L.A. Lione, Diabetic dyslipidaemia is associated with alterations in eNOS, caveolin-1, and endothelial dysfunction in streptozotocin treated rats, Diabetes/metabolism research and reviews, (2018) e2995.
[22] E. Manders, F. Verbeek, J. Aten, Measurement of co-localization of objects in dual-colour confocal images, Journal of microscopy, 169 (1993) 375-382.
[23] X.-Y. Qi, J.G. Diness, B. Brundel, X.-B. Zhou, P. Naud, C.-T. Wu, H. Huang, M. Harada, M. Aflaki, D. Dobrev, Role of small conductance calcium-activated potassium channels in atrial electrophysiology and fibrillation in the dog, Circulation, (2013) CIRCULATIONAHA. 113.003019.
[24] X. Zhou, N. Voigt, T. Wieland, D. Dobrev, Enhanced frequencydependent retograde trafficking of small conductance Ca2+-activated K+ channels may contribute to electrical remodeling in human atrial fibrillation, Heart Rhythm, 9 (2012) S319.
[25] J. Heijman, N. Voigt, S. Nattel, D. Dobrev, Cellular and molecular electrophysiology of atrial fibrillation initiation, maintenance, and progression, Circulation research, 114 (2014) 1483-1499.
[26] Y. Xu, D. Tuteja, Z. Zhang, D. Xu, Y. Zhang, J. Rodriguez, L. Nie, H.R. Tuxson, J.N. Young, K.A. Glatter, Molecular identification and functional roles of a Ca2+-activated K+ channel in human and mouse hearts, Journal of Biological Chemistry, (2003).
[27] M.M. Haugaard, E.Z. Hesselkilde, S. Pehrson, H. Carstensen, M. Flethøj, K.F. Præstegaard, U.S. Sørensen, J.G. Diness, M. Grunnet, R. Buhl, Pharmacologic inhibition of small-conductance calcium-activated potassium (SK) channels by NS8593 reveals atrial antiarrhythmic potential in horses, Heart Rhythm, 12 (2015) 825-835.
[28] J.E. Kirchhoff, J.G. Diness, L. Abildgaard, M. Sheykhzade, M. Grunnet, T. Jespersen, Antiarrhythmic effect of the Ca 2+-activated K+(SK) channel inhibitor ICA combined with either amiodarone or dofetilide in an isolated heart model of atrial fibrillation, Pflügers Archiv-European Journal of Physiology, 468 (2016) 1853-1863.
[29] L. Skibsbye, A.K. Bengaard, A. Uldum-Nielsen, K. Boddum, T. Christ, T. Jespersen, Inhibition of Small Conductance Calcium-activated Potassium (SK) Channels prevents Arrhythmias in Rat Atria during β-adrenergic and Muscarinic receptor activation, Frontiers in Physiology, 9 (2018).
[30] M. Grunnet, B.H. Bentzen, U.S. Sørensen, J.G. Diness, Cardiac ion channels and mechanisms for protection against atrial fibrillation, Reviews of physiology, biochemistry and pharmacology, Springer2011, pp. 1-58.
[31] J.-F. Renaud, C. Desnuelle, H. Schmid-Antomarchi, M. Hugues, G. Serratrice, M. Lazdunski, Expression of apamin receptor in muscles of patients with myotonic muscular dystrophy, Nature, 319 (1986) 678.
[32] M. Favero, D.J. Jiang, C. Chiamulera, A. Cangiano, G.F. Fumagalli, Expression of small-conductance calcium-activated potassium channels (SK3) in skeletal muscle: regulation by muscle activity, The Journal of physiology, 586 (2008) 4763-4774.

| | **Total (N=18)** |
|---|---|
| **Age in years, median (IQR)** | 70 (64.3, 72.5) |
| **Male gender, n (%)** | 15/18 (83.3) |
| **History of atrial fibrillation at consent, n (%)** | 5/18 (27.8) |
| **NYHA Score, n (%)** | |
| I | 2/17 (11.8) |
| II | 7/17 (41.2) |
| III | 8/17 (47.1) |
| **Smoking history, n (%)** | |
| Current smoker | 1/18 (5.6) |
| Ex smoker >1 month | 5/18 (27.8) |
| **Diabetes, n (%)** | |
| Type II | 4/18(22.2) |
| **Medical history, n (%)** | |
| Hypertension requiring treatment | 12/18 (66.7) |
| Myocardial infarction | 3/18 (16.7) |
| Hypercholesterolaemia | 13/18 (72.2) |
| Congestive heart failure | 1/18 (5.6) |
| **Pre-operative medications, n (%)** | |
| Aspirin | 8/18 (44.4) |
| Clopidogrel | 2/18 (11.1) |
| Warfarin | 2/18 (11/1) |
| Beta blockers | 9/18 (50.0) |
| Angiotensin II blockers | 4/18 (22.2) |
| Calcium antagonists | 4/18 (22.2) |
| Diuretics | 8/18 (44.4) |
| Statins | 11/18 (61.1) |
| **Pre-operative haemoglobin (g/l), median (IQR)** | 142 (140.3, 152.0) |
| **Pre-operative ECG results, n (%)[$]** | |
| Sinus rhythm | 12/18 (66.7) |
| Atrial fibrillation | 3/18 (16.7) |
| Atrioventricular block | 3/18 (16.7) |
| Other^ | 1/18 (5.6) |
| **Pre-operative LV function, n (%)** | |
| Good (EF >60%) | 14/18 (77.8) |
| Mildly impaired (EF 50-60%) | 3/18 (16.7) |
| Moderately impaired (EF 30-49%) | 1/18 (5.6) |

IQR: interquartile range; NYHA: New York heart association; ECG: electrocardiogram; LV: left ventricular.

[$] More than one result could be selected per patient. ^ Sinus bradycardia (n=1); sinus bradycardia with premature atrial complexes (n=1).

**Table 1: Baseline demographics and clinical details of consented participants whose samples are included in analyses**

33 patients consented, while 31 received collection, which were assessed successfully in 18 cases.

**Figure 1 Inhibition of SK channel current in whole-cell voltage-clamped human atrial myocytes. Ai**. Membrane current evoked by a step depolarization to 10 mV from a holding potential of -80 mV. Application of apamin (100 nM) (grey trace) reduced the amplitude of evoked control current (black trace), with the initial fast inward sodium current partially curtailed. **Aii**. Normalised current-voltage relationship of current evoked by step depolarization from a holding potential of -80 mV (●). Application of apamin (100 nM) significantly reduced the amplitude of evoked current at potentials positive to -10 mV (○) ($P<0.05$ at -20 mV, $P<0.0001$ at -10 to +10 mV). Inset shows the current-voltage relationship of apamin-sensitive current, with apamn inhibiting current positive to -50 mV. **Bi**. Outward current evoked by a step depolarization to 10 mV from a holding potential of -80 mV (black trace) was reduced by UCL1684 (100 nM) (grey trace). **Bii**. Current-voltage relationship for current evoked by step depolarization from a holding potential of -80 mV in the absence (●) and presence of UCL1684 (100 nM) (○). The SK current inhibitor significantly inhibited current positive to -30 mV ($P<0.05$ at -30 mV, $P<0.01$ at -20 mV, $P<0.0001$ at -10 to +10 mV). Inset shows the current-voltage relationship for the current inhibited by UCL1684 (100 nM).

**Figure 2. Atypical pharmacology of SK channel current underlying the atrial action potential. A**. Action potential voltage command waveform delivered to voltage clamped human atrial myocytes. This was generated by the Courtemanche et al human atrial AP model (Am J Physiol, 1998, 275, H301-H321). **B:** Difference current between control and apamin containing solutions from a human atrial myocyte voltage clamped -80 mV, to which the AP command was applied. The trace profile shows little effect of apamin (100 nM). **C:** UCL1684 (100 nM)-sensitive action potential currents evoked by subtraction of residual (UCL1684-insensitive) current from control current (different cell from B). There was a clear outward UCL 1684-sensitive current during the applied AP command. The waveform demonstrates that activation of a UCL1684-sensitive current occurs early in the action potential waveform, which declines during the voltage command.

**Figure 3. Presence of SK2 and SK3 channel subunits in human atrial myocytes. A**. Confocal images (0.5-μm section) of a fixed and permeabilized cell labelled with anti-SK2 (green) and anti-SK3 (red). Labelling was observed at the cell periphery and superimposition of both images showed a significant degree of colocalization (yellow). **B**. Graph illustrates that an apparent similar level of labelling of SK2 and SK3 subunits in acutely isolated human atrial myocytes. **C**. Confocal images of SK2 (green) and SK3 (red) showing labelling at the cell edge. Superimposition of SK2 and SK3 channel subunit labelling demonstrates significant colocalization (yellow), which was quantified by using Mander's coefficient. Measured SK2 and SK3 labelling gave a Mander's coefficient of co-localization of SK3 overlapping SK2 by 0.94 ± 0.05 and SK2 overlapping SK3 by 093 ± 0.03.

**Figure**

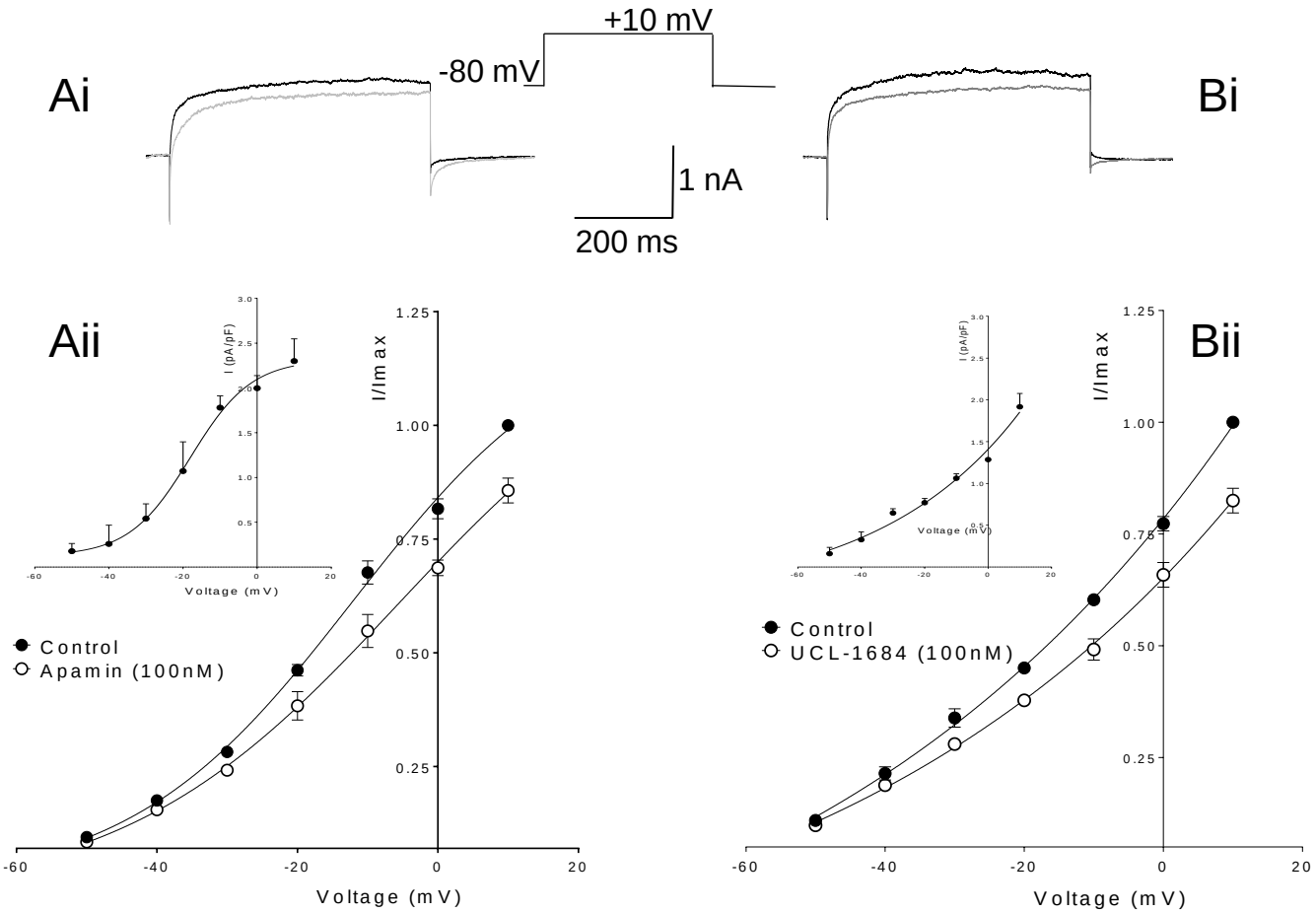


Figure 1. Shamsaldeen et al

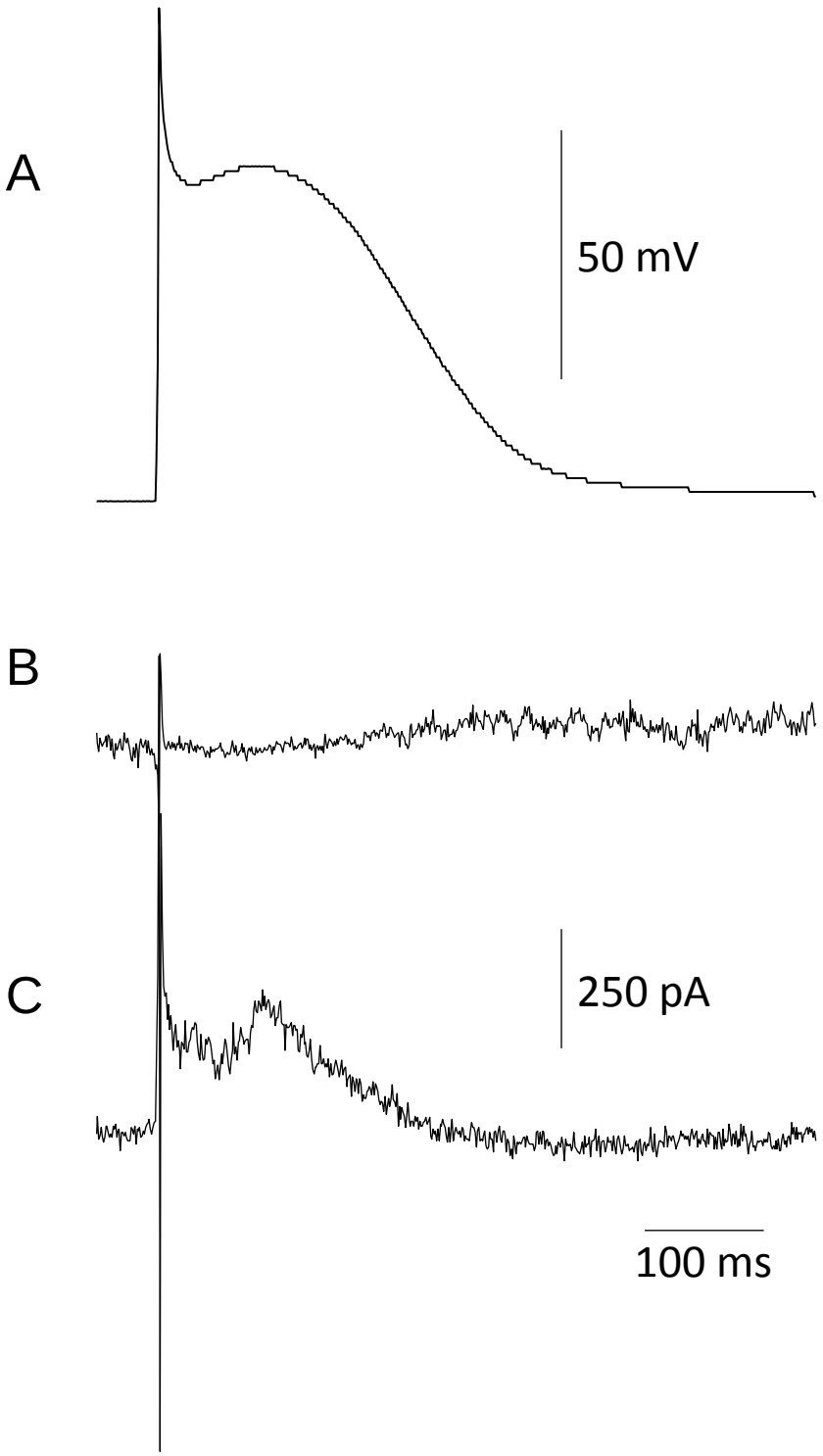


Figure 2. Shamsaldeen et al

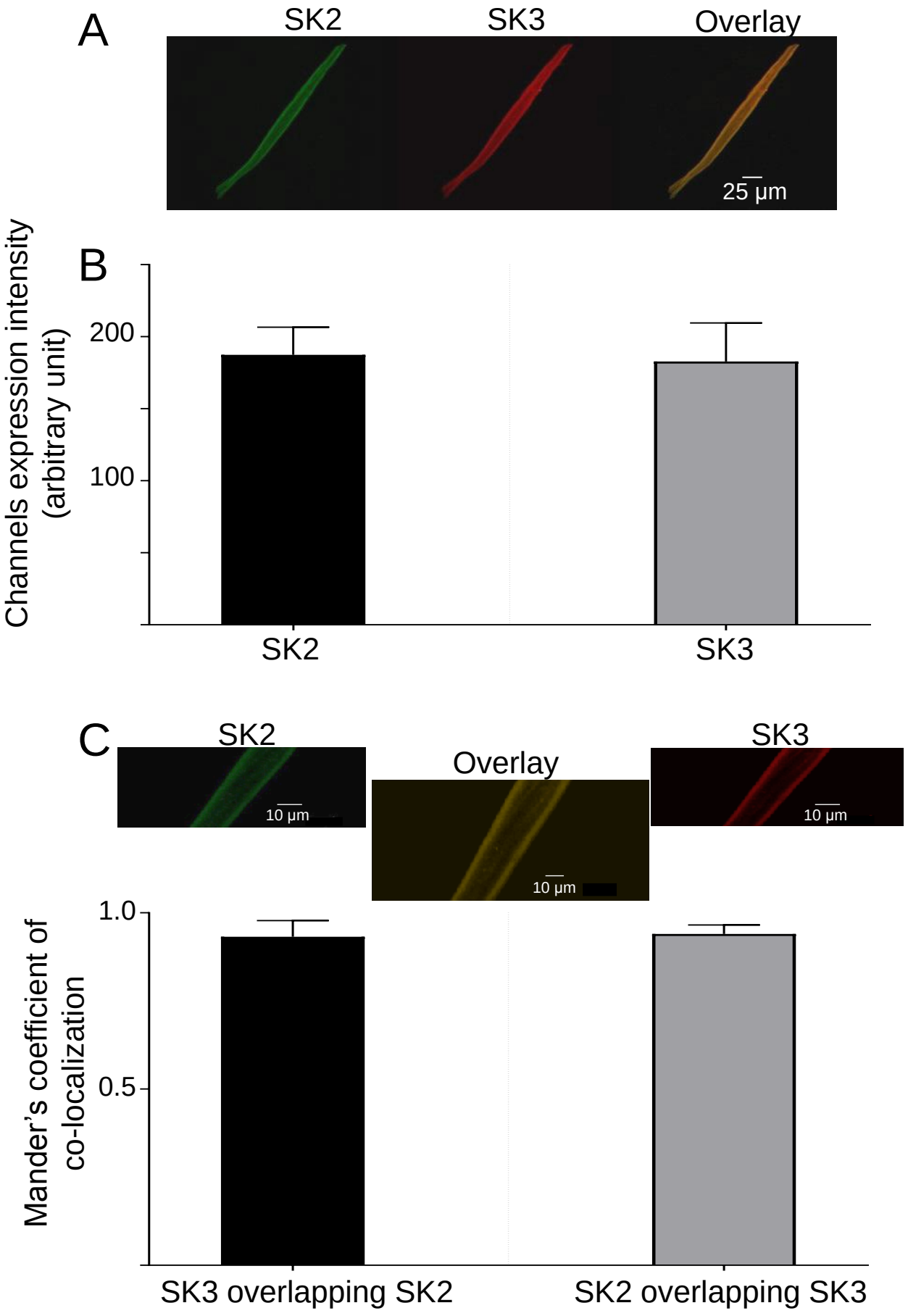


Figure 3. Shamsaldeen et al